Inactivation of *Listeria monocytogenes* in Skim Milk and Liquid Egg White by Antimicrobial Bottle Coating with Polylactic Acid and Nisin

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ABSTRACT: This study was to develop an antimicrobial bottle coating method to reduce the risk of outbreaks of human listeriosis caused by contaminated liquid foods. Liquid egg white and skim milk were inoculated with *Listeria monocytogenes* Scott A and stored in glass jars that were coated with a mixture of polylactic acid (PLA) polymer and nisin. The efficacy of PLA per nisin coating in inactivating *L. monocytogenes* was investigated at 10 and 4 °C. The pathogen grew well in skim milk without PLA/nisin coating treatments, reaching 8 log CFU/mL after 10 d and then remained constant up to 42 d at 10 °C. The growth of *Listeria* at 4 °C was slower than that at 10 °C, taking 21 d to obtain 8 log CFU/mL. At both storage temperatures, the PLA coating with 250 mg nisin completely inactivated the cells of *L. monocytogenes* after 3 d and throughout the 42-d storage period. In liquid egg white, *Listeria* cells in control and PLA coating without nisin samples declined 1 log CFU/mL during the first 6 d at 10 °C and during 28 d at 4 °C, and then increased to 8 or 5.5 log CFU/mL. The treatment of PLA coating with 250 mg nisin rapidly reduced the cell numbers of *Listeria* in liquid egg white to undetectable levels after 1 d, then remained undetectable throughout the 48 d storage period at 10 °C and the 70 d storage period at 4 °C. These data suggested that the PLA/nisin coating treatments effectively inactivated the cells of *L. monocytogenes* in liquid egg white and skim milk samples at both 10 and 4 °C. This study demonstrated the commercial potential of applying the antimicrobial bottle coating method to milk, liquid eggs, and possibly other fluid products.

Keywords: antimicrobial bottle coating, *L. monocytogenes*, liquid egg, milk, nisin, PLA

Introduction

Recent outbreak of *Listeria monocytogenes* infections associated with pasteurized milk in Massachusetts raised more public concerns over the safety of dairy foods. In 2007, 5 cases of listeriosis were identified, and 3 deaths occurred in residents of central Massachusetts. The subsequent investigation found that pasteurized, flavored and nonflavored, fluid milk produced by a local dairy were the source of the outbreak. This outbreak illustrated the potential for contamination of fluid milk products after pasteurization (CDC 2008). In 1983, another outbreak was caused by contaminated pasteurized whole or 2% fat milk in Massachusetts, claiming at least 14 lives (Fleming and others 1985). In 1985, a contaminated Mexican-style soft cheese was reported to be the carrier of *L. monocytogenes* in milk that caused another outbreak of human listeriosis in the state of California. There were more than 100 confirmed cases in this outbreak and at least 40 people died (James and others 1985).

In a survey conducted by the United States Food and Drug Administration (1986) beginning in 1986, 2.5% of 357 dairy processing plants were found to have products containing *L. monocytogenes* (Prentice and Neaves 1988). The presence of *L. monocytogenes* in the final product was thought to be due to faulty thermal treatment or postpasteurization contamination, mainly through contact with raw milk (Saan and others 1993). However, several studies have reported the *L. monocytogenes* could survive pasteurization (Fleming and others 1985; Doyle and others 1987; Louett and others 1987). Cells of *L. monocytogenes* have been isolated from commercially processed liquid whole egg in the United States (Leasor and Foegeding 1989) and Northern Ireland (Moore and Madden 1993). Although no outbreaks of listeriosis have been attributed to eggs, the potential exists for survival and growth of *L. monocytogenes* in egg products.

L. monocytogenes can grow at temperatures of 4 to 45 °C (Doyle and Beuchat 2007), and refrigeration alone is not sufficient to prevent its growth in foods. Its presence in raw and certain processed foods may be unavoidable (WHO 1988). Following the packaging process, no other antimicrobial intervention is utilized other than refrigeration. Therefore, a secondary preservative treatment is desirable. Antimicrobial packaging provides an additional and final barrier that can prevent the growth of foodborne pathogens.

In recent years, there has been a growing interest to use natural antimicrobials, especially nisin, in food packaging applications. Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*. It is effective against gram-positive bacteria and ineffective against fungi and gram-negative bacteria (Jay 1996). Nisin was affirmed generally recognized as safe (GRAS) by the Food and Drug Administration (1988) in 1988, and is now used as a biopreservative in 57 countries around the world. Up to 400 units/g food is usually recommended for food preservation (Hurst and Hoover 1993). Because it is nontoxic, heat stable, and does not contribute to off-flavors, nisin is commercially used in a variety of foods

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including dairy, eggs, vegetables, meat, fish, beverages, and cereal-based products to inhibit growth of foodborne pathogens including *Listeria monocytogenes* (Schillinger and others 1996).

Nisin incorporated into polyethylene or other polymer films alone or with other antimicrobial agents, was effective against various microorganisms, including *L. plantarum*, *L. monocytogenes*, *E. coli*, and *Salmonella* spp. (Padgett and others 1998; Cutter and others 2001; Eswaranandam and others 2004). A variety of polymer films have been used to deliver nisin. Some examples of these polymer films include: sodium caseinate films (Kristo and others 2008), glucomannan–gellan gum blend films (Xu and others 2007), alginate films (Natrajan and Sheldon 2000a; Cha and others 2002; Millette and others 2007), glucomannan–chitosan films (Li and others 2006), methylcellulose/hydroxypropyl methylcellulose films (Franklin and others 2004), poly(vinyl chloride), linear low-density polyethylene and nylon films (Natrajan and Sheldon 2000b), corn zein films (Hoffman and others 2001; Janes and others 2002), whey protein, soy protein, egg albumin, and wheat gluten films (Ko and others 2001).

Poly (lactic acid) (PLA) is a biodegradable and compostable polymer that can be derived from renewable resources. Polymers of PLA are of current interest not only because of the need to reduce the use of many fossil fuel-derived polymers but also due to the growing global problems associated with plastic waste disposal (Plackett and others 2006). The use of PLA in food packaging has already received wide attention (Conn and others 1995; Sinclair 1996; Haugaard and others 2002; Frederiksen and others 2003). A few PLA-based containers, such as Biota™ PLA bottled water, Noble™ PLA bottled juices and Dannon™ yogurts, have been made available in market. However, limited information is available for using PLA polymers as a carrier of nisin. Salmasoa and others (2004) loaded nisin into PLA particles and evaluated the sustained antimicrobial activity of nisin from the nisin/PLA particles. However, neither a film form of nisin/PLA nor pathogens in a food system were investigated in their study. To our knowledge, there is no literature available using PLA as a carrier for bottle coating in food applications.

Our previous studies demonstrated that nisin incorporated in PLA or pectin/PLA film retained more activity against *L. monocytogenes* over a 48 h period than a direct addition of a similar total dose and suggested that the incorporation of nisin into the PLA polymer could provide a possible delivery system for improving the efficacy of nisin in food applications (Jin and Zhang 2008; Jin and others 2009a, 2009b). The objective of this project was to extend our previous studies to develop an antimicrobial packaging system for liquid foods to inactivate food borne pathogens which may survive thermal pasteurization or come from postpasteurization contamination. In this study, *L. monocytogenes* and liquid egg and milk were used as a model for evaluating the efficacy of PLA per nisin bottle coating in inactivation of the pathogen in liquid foods.

Materials and Methods

Food samples

Skim milk with 0.3% fat and liquid egg white were used for this experiment. Commercial pasteurized skim milk and liquid egg white without preservatives were purchased from a local grocery store. The pHs of skim milk and liquid egg white were 6.8 and 8.2, respectively.

Culture

Listeria monocytogenes Scott A 724 was obtained from the culture collection of the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center. Stock cultures were maintained at −80 °C. The inoculating cultures were propagated on Tryptic Soy Agar (TBA) (Difco/Becton Dickinson, Sparks, Md., U.S.A.) at 37 °C and maintained at 0 to 2 °C until use. Prior to the inoculum preparation, *L. monocytogenes* cells were grown in brain heart infusion broth (BHIB) (Difco/Becton Dickinson) aerobically at 37 °C for 16 to 18 h.

Antimicrobial coating

One gram of PLA resin (4060D, Natureworks, Minnetonka, Mich., U.S.A.) and 80 to 500 mg nisin powder (2.5% purity, Sigma Chemical Co., St. Louis, Mo., U.S.A.) were accurately weighed and dispersed in 15 mL of methylene chloride. This mixture was stirred by a magnetic bar until the polymer was totally dissolved and nisin powder was evenly dispersed into the polymer. The mixture was transferred into precleaned 4 oz glass jars (Chases Scientific Glass, Rockwood, Tenn., U.S.A.), which were horizontally rolling so the mixture could coat the inside wall of the jars. The methylene chloride was evaporated at room temperature during the jar's rolling under a chemical hood. The coated jars were dried in a vacuum oven in an upright position at 90 °C for 12 h, and then sealed with lids and stored until time of use. In the screen tests, 20, 80, 125, 250, and 500 mg nisin powders were used. The maximal concentrations of nisin, if fully released into 100 mL of test medium, were equivalent to 20, 80, 125, 250, and 500 IU/mL, respectively. For other tests, 250 mg nisin powder was used.

Antibacterial test

For coating/release test, coated 4 oz glass jars contained 100 mL of liquid medium (milk or liquid egg) that was inoculated with overnight cultures of *L. monocytogenes*. The final cell density in the medium was approximately 1×10^4 cells/mL in each test. The jars were shaken at 50 rpm at 4 or 10 °C. The inoculated media were sampled (1 mL) at several time intervals (2, 3, 4, or 7 d). Specimens were serially diluted by sterile Butterfield's phosphate buffer (pH 7.2, Hardy Diagnostics, Santa Maria, Calif., U.S.A.), and then pour plated onto *Listeria*-specific Palcam Agar (Difco/Becton Dickinson) with Palcam selective supplement (Oxoid, Hampshire, England). Plates were incubated at 37 °C for 24 h. An inoculated medium without coating treatment served as a control. The storage temperature was tested at 10 °C, which is an abusive refrigeration temperature and at 4 °C, which is the ideal storage temperature according to manufacturers' suggestion during distribution, retail, or home storage. Each experiment was conducted in triplicate.

Statistical analysis

Antimicrobial experiments were conducted in triplicate. Data points were expressed as the mean ± SD. Data were analyzed using analysis of variance (ANOVA) from SAS version 9.1 software (SAS Inst., Cary, N.C., U.S.A.). Duncan's multiple range tests were used to determine the significant difference of mean values. Unless stated otherwise, significance was expressed at 5% level.

Results and Discussion

The average thickness of the PLA coating films was 0.15 mm. The addition of nisin to PLA coatings had no effect on the coating thickness or the variation of coating thickness compared with the PLA coating only (data not shown). However, addition of nisin to PLA did affect the optical properties of the coating; the glass jars changed from transparent to semitransparent depending on the amount of nisin powder used as shown in Figure 1. Figure 1A shows the pictures of coated glass jars before used for antimicrobial tests. The change of optical property of PLA coatings was mainly

due to the nisin powder that contains 97.5% of milk protein and salt. Hence, use of pure nisin in PLA coating could improve the optical property, which needs to be further investigated. There was no delamination observed after exposure of the coatings to milk sample for 42 d at 10 °C (Figure 1B). Similar results were observed for the coated jars containing liquid egg white samples (pictures not shown).

The 1st experiment for antimicrobial tests was to incorporate varying amounts (20, 80, 125, 250, and 500 mg per coating) of nisin into the PLA coatings and to determine the appropriate amount of nisin concentration for coating formation and its antimicrobial activity in skim milk samples. The antimicrobial activities of PLA/nisin coatings against *L. monocytogenes* are listed in Tables 1 and 2. The cell population of *L. monocytogenes* in control and PLA coating samples reached 8 log CFU/mL after 10 d at 10 °C (Table 1) or 28 d at 4 °C (Table 2). The PLA coating without nisin did not contribute any antilisterial activity. As the nisin concentration increased, the inhibitory effect progressively increased, but there was no significant difference between 20 mg nisin coating and controls at both 4 and 10 °C. The coating treatment containing 250 mg nisin significantly reduced the cell concentration to 1.2 approximately 1.6 log CFU/mL at 1 d then reduced to undetectable levels after 3 d and over the 28-d storage period. The similar phenomenon was observed with the coating treatment containing 500 mg nisin at both storage temperatures and also for liquid egg white samples (data not shown). Therefore, PLA coating with 250 mg nisin was selected for subsequent experiments.

Figure 1 — Coated glass jars with PLA + 250 mg nisin powder (left) and PLA only (right). A: coated jars before antimicrobial test; B: coated jars with milk after 42 d at 10 °C.

Based on the previous results, PLA coatings with 250 mg nisin were used to treat skim milk samples inoculated with *L. monocytogenes*. In this experiment, the storage time was extended to 42 d at both 10 and 4 °C (Figure 2). Repeatedly, *L. monocytogenes* grew well in skim milk in the absence of nisin; the organism rapidly reached 8 log CFU/mL after 10 d and then remained through to 42 d at 10 °C (Figure 2A). The growth of *Listeria* at 4 °C was slower than that at 10 °C; it took 21 d to reach 8 log CFU/mL (Figure 2B). At both storage temperatures, the PLA coating with 250 mg nisin completely inactivated the cells of *L. monocytogenes* after 3 d and throughout the 42 d storage period. No *Listeria* cell recovery was observed by the end of storage.

The same PLA coating with 250 mg nisin was used to treat *L. monocytogenes* in liquid egg white. Figure 3 shows the survivals of *L. monocytogenes* in liquid egg white samples in the absence or presence of PLA/nisin coating during storage at 10 °C (A) and 4 °C (B). Similar to milk samples, the PLA coating without nisin had little or no effect on suppressing the growth of *L. monocytogenes* in liquid egg white, as expected.

After an initial 1 log CFU/mL drop in population of *Listeria* during the first 6 d of storage at 10 °C, which may be due to the adaption of cells to new environment, cell populations were increased to 5.5, 7.7, and 8.1 log CFU/mL by day 13, 20, and 42, respectively. The treatment of PLA per nisin coating reduced the cells to undetectable levels by day 1 and the cell concentrations remained at those levels throughout the rest of storage period (Figure 3A). When 4 log CFU/mL cells of *L. monocytogenes* were inoculated in liquid egg white, the cells in PLA coating and control samples gradually decreased during the storage at 4 °C and declined to 3 log CFU/mL by day 42 (Figure 3B). The cell concentration then increased to 5.5 log CFU/mL by day 70. Similar to samples stored at 10 °C, the treatments of PLA per nisin coatings significantly affected

Table 1 — Survivals (log CFU/mL) of *Listeria monocytogenes* in skim milk during storage at 10 °C.[a]

	Coating treatment						
Time (d)	Control	PLA	PLA + 20 mg nisin	PLA + 80 mg nisin	PLA + 125 mg nisin	PLA + 250 mg nisin	PLA + 500 mg nisin
0	3.35 ± 0.17a	3.35 ± 0.17a	3.35 ± 0.17a	3.35 ± 0.17a	3.35 ± 0.17a	3.35 ± 0.17a	3.35 ± 0.17a
1	3.51 ± 0.25a	3.58 ± 0.19a	3.34 ± 0.18a	3.01 ± 0.14b	2.51 ± 0.19c	1.21 ± 0.23d	0.63 ± 0.18e
3	4.34 ± 0.15a	4.26 ± 0.16a	4.08 ± 0.15b	3.33 ± 0.21c	2.85 ± 0.24d	UD e	UD e
7	7.05 ± 0.19a	6.99 ± 0.21a	6.46 ± 0.16b	4.61 ± 0.25c	2.79 ± 0.33d	UD e	UD e
10	8.21 ± 0.23a	8.12 ± 0.32a	7.68 ± 0.19a	5.30 ± 0.18b	3.65 ± 0.22c	UD d	UD d
14	8.18 ± 0.18a	8.01 ± 0.25a	7.67 ± 0.27a	5.91 ± 0.15b	4.56 ± 0.21c	UD d	UD d
17	8.21 ± 0.19a	8.23 ± 0.15a	7.82 ± 0.19b	6.77 ± 0.20c	5.41 ± 0.15d	UD e	UD e
21	8.13 ± 0.22a	8.14 ± 0.24a	8.08 ± 0.19a	7.20 ± 0.14b	6.02 ± 0.25c	UD d	UD d
24	8.70 ± 0.31a	8.39 ± 0.22a	8.21 ± 0.25a	7.54 ± 0.17b	6.51 ± 0.18c	UD d	UD d
28	8.38 ± 0.15a	8.33 ± 0.18a	8.22 ± 0.17a	7.81 ± 0.15b	7.02 ± 0.31c	UD d	UD d

[a]Data are presented as the mean values of 3 replications ± SD. Means within each row that have a common letter are not significantly different ($P > 0.05$).
UD = undetectable (< 1 CFU/mL).

the survival of *L. monocytogenes*. The PLA coatings containing nisin reduced the number of *L. monocytogenes* to undetectable populations at day 1 and afterwards.

Our results demonstrated that nisin in PLA coating effectively reduced and inactivated *L. monocytogenes* in skim milk and liquid egg white when 250 mg or more nisin (500 mg) was used. Bhatti and others (2004) observed that *L. monocytogenes* strain Scott A was most sensitive to nisin in skim milk, showing rapid decline in cell numbers to less than 10 CFU/mL after 9 h at 5 °C following treatment with ≥ 250 IU/mL nisin. Orr and others (1998) reported that refrigerated milk samples inoculated with *L. monocytogenes* had a reduction of approximately 2 logs when exposed for 48 h to cast corn zein films containing nisin. It was also found by Kim and others (2008) that nisin at 62.5, 125, 250, and 500 IU/mL inhibited the growth of *L. monocytogenes* in skim milk, and nisin at 250 and 500 IU/mL reduced the initial cell count numbers for 2 d with no cell growth being observed until 10 d.

With respect to liquid egg products, similar results have also reported. Our previous study (Jin and others 2009b) showed that the cell population of *L. monocytogenes* in liquid egg white with PLA/pectin + nisin film (approximately 1000 IU nisin per milliliter of liquid egg white) was reduced from 6.8 to 2 log CFU/mL while the control remained at 6.5 log CFU/mL after 48 h. Similarly, Delves-Broughton and others (1992) reported that the addition of nisin at 200 IU/mL extended the shelf life of conventionally pasteurized liquid whole egg at 6 °C by 9 to 11 d relative to nisin-free control samples. Schuman and Sheldon (2003) demonstrated that *Listeria*

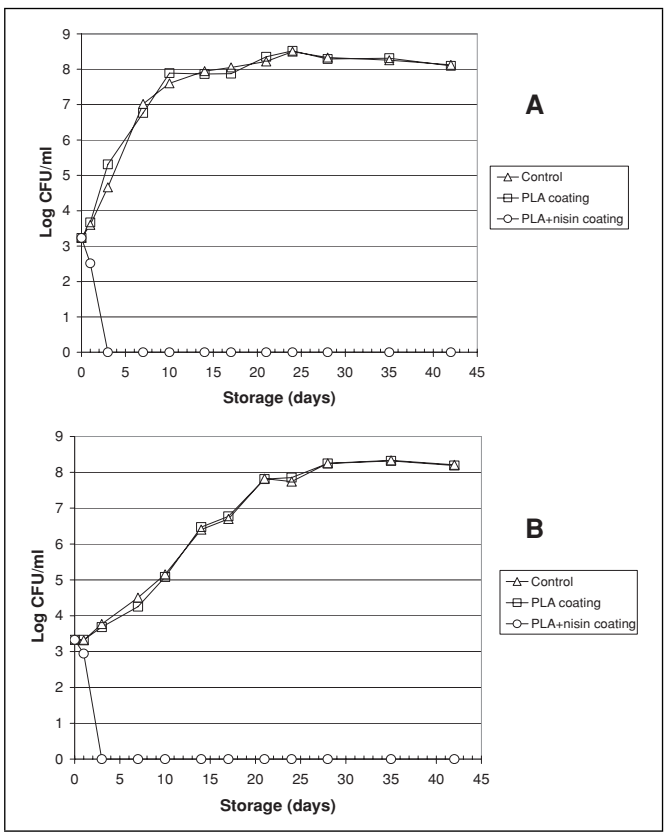

Figure 2—Effect of PLA/nisin coating treatment on *Listeria monocytogenes* in skim milk at 10 °C (A) and 4 °C (B). 250 mg of nisin was incorporated into PLA coating.

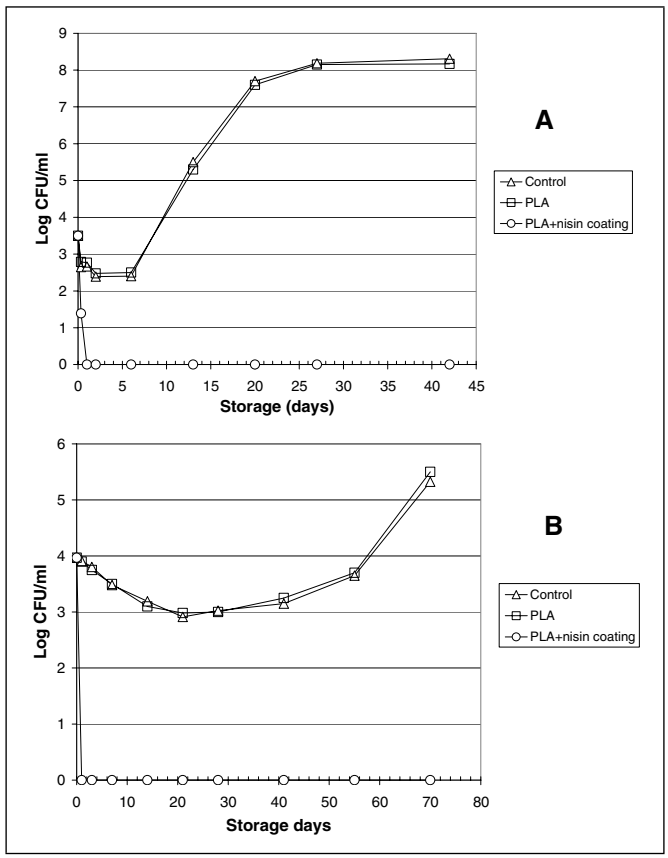

Figure 3—Effect of PLA/nisin coating treatment on *Listeria monocytogenes* in liquid egg white at 10 °C (A) and 4 °C (B). 250 mg of nisin was incorporated into PLA coating.

Table 2—Survivals (log CFU/mL) of *Listeria monocytogenes* in skim milk during storage at 4 °C.[a]

	Coating treatment						
Time (d)	Control	PLA	PLA + 20 mg nisin	PLA + 80 mg nisin	PLA + 125 mg nisin	PLA + 250 mg nisin	PLA + 500 mg nisin
0	3.31 ± 0.15a	3.31 ± 0.15a	3.31 ± 0.15a	3.31 ± 0.15a	3.31 ± 0.15a	3.31 ± 0.15a	3.31 ± 0.15a
1	3.32 ± 0.18a	3.30 ± 0.25a	3.34 ± 0.19a	3.01 ± 0.11b	2.49 ± 0.13c	1.67 ± 0.17d	1.32 ± 0.16e
3	3.77 ± 0.17a	3.70 ± 0.22a	3.50 ± 0.11a	3.33 ± 0.14b	2.55 ± 0.15c	UD d	UD d
7	4.50 ± 0.12a	4.40 ± 0.25a	4.11 ± 0.15b	3.50 ± 0.21c	2.76 ± 0.14d	UD e	UD e
10	5.16 ± 0.19a	5.10 ± 0.26a	4.80 ± 0.21a	3.90 ± 0.11b	3.00 ± 0.23c	UD d	UD d
14	6.30 ± 0.17a	6.20 ± 0.18a	5.92 ± 0.20a	4.90 ± 0.15b	3.41 ± 0.15c	UD d	UD d
17	6.70 ± 0.15a	6.80 ± 0.14a	6.71 ± 0.17a	5.91 ± 0.25b	4.50 ± 0.13c	UD d	UD d
21	7.62 ± 0.23a	7.72 ± 0.15a	7.62 ± 0.11a	6.52 ± 0.19b	4.91 ± 0.15c	UD d	UD d
24	7.74 ± 0.16a	7.83 ± 0.19a	7.70 ± 0.18a	6.90 ± 0.15b	5.62 ± 0.21c	UD d	UD d
28	8.25 ± 0.24a	8.28 ± 0.25a	8.02 ± 0.19a	7.51 ± 0.17b	6.81 ± 0.26c	UD d	UD d

[a]Data are presented as the mean values of 3 replications ± SD. Means within each row that have a common letter are not significantly different ($P > 0.05$).
UD = undetectable (< 1 CFU/mL).

reductions of 3 log CFU/mL were achieved within 24 h in liquid whole egg with nisin (500 IU/mL); in liquid whole egg containing 0 and 100 IU/mL nisin, logarithmic growth of *L. monocytogenes* had begun within 7 d at 4 °C, and maximum populations of 6 log CFU/mL were achieved within 4 wk. Motlagh and others (1991) stated a mean reduction of 3.4 log cycles for 9 strains of *L. monocytogenes* individually exposed to Nisaplin (500 IU of nisin per milliliter) for 24 h at 4 °C.

The growth pattern of *L. monocytogenes* in this study (Table 2, Figure 2B and 3B) confirmed that milk and liquid egg support *Listeria* growth under refrigerated storage conditions. Lower storage temperature can slow the growth of *L. monocytogenes* but cannot prevent growth of *L. monocytogenes* in milk or liquid egg white. It took 21 d to reach 8 log CFU/mL at 4 °C, compared to 10 d at 10 °C in milk samples. Similarly, *Listeria* cells in liquid egg white took 55 d at 4 °C or 6 d at 10 °C for adaptation to the environment before rapid regrowth. The mean minimum growth temperature of *L. monocytogenes* was found to be 1.1 °C with a range of 0.5 to 3 °C (Junttila and others 1988). Previous researches have shown that *L. monocytogenes* may attain maximum populations of 6 to 7 log CFU/mL within approximately 3 wk in ultrapasteurized liquid egg white (Foegeding and Leasor 1990) and that high rates of growth of *L. monocytogenes* were found at refrigeration temperatures in pasteurized milk within its shelf life (FDA 2003).

It is interesting to note that at both storage temperatures *Listeria* in liquid egg samples in the absence of nisin (control and PLA coating) had a longer lag phase than that in milk samples. The characteristics of milk (pH close to neutrality, large presence of nutrients) could have favored the increase of the viable counts of *L. monocytogenes*. An alternative explanation may be the presence of lysozyme, a natural antimicrobial compound in eggs. Lysozyme is known to be bactericidal to gram-positive microorganisms through hydrolysis of the 1-4 glycosidic bonds between *N*-acetyl glucosamine and *N*-acetyl muramic acid in the peptidoglycan layer of the bacterial cell wall (Masuda and others 2001). Lysozyme may play a role in extending the lag phase of *Listeria* growth in liquid egg white because actual commercial pasteurization conditions for liquid eggs do not inactivate lysozyme (Durance 1994). Although lysozyme also occurs naturally in milk at a concentration of approximately 0.13 μg/mL (Reiter 1978), it could be insignificant as compared to 2250 approximately 3270 μg lysozyme/mL in egg white (Arora and others 1974).

Antimicrobials can be added to food formulations directly or through slow release from packaging materials. Direct addition of antimicrobials into foods results in an immediate reduction of bacterial populations but may not prevent the recovery of injured cells or the growth of cells that were not destroyed by direct addition if residues of the antimicrobial are rapidly depleted (Zhang and others 2004). The application of antimicrobial coatings allows for the migration of the antimicrobial to the coating surface and provides a continuous antimicrobial effect on the food during extended exposure. In our previous study (Jin and Zhang 2008), we demonstrated that the retention of nisin activity occurred when nisin was incorporated into a PLA polymer and the PLA per nisin polymer exhibited effective antibacterial activity against foodborne *L. monocytogenes*, *E. coli* O157:H7 and *S. Enteritidis*. Use of polymers as carriers of antimicrobials such as nisin not only permits controlled release of these antimicrobials but also prevents dramatic reductions in their antimicrobial activities due to their affinity for food particles and inactivation by components in foods. Therefore, the use of antimicrobial packaging such as bottles/jar coatings can offer advantages, compared with the direct addition of preservatives to the liquid foods since only low levels of preservative come into contact with the food. These data indicated that PLA bottle coatings can act as suitable carriers for delivering effective antimicrobials to food products.

The high thermal sensitivity of liquid egg components prevents the application of more intense heat treatments to inactivate pathogens. The dairy industry is also developing minimum processing techniques (Goff and Griffiths 2006) that can be used to prolong the shelf life and improve the sensorial characteristics of fluid milk. Therefore, it would be desirable to have an additional hurdle for improving the safety of liquid food products. Antimicrobial active bottle coatings represent an innovative concept in food packaging, developed to answer to consumer's expectation for better microbiological safety.

Conclusions

The results of the present study allow us to conclude that *L. monocytogenes* can grow to high levels on skim milk and liquid egg white, even at normal refrigeration temperature and especially at abusive temperature. Additionally, the PLA per nisin coating treatments effectively inactivated the cells of *L. monocytogenes* in these foods at both temperatures. The application of PLA and nisin based antimicrobial packaging in food system is very encouraging. With the recurring recalls of foods due to contamination with food borne pathogens, there is a commercial potential of applying such antimicrobial bottle coating methods to milk, liquid eggs, and possibly other juice and beverage products to inactivate *Listeria* or other pathogens.

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